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An Insecticidal and Acaricidal Polysulfide Metabolite from the Roots of *Petiveria alliacea*

Lyndon Johnson,[1] Lawrence A. D. Williams[2]* and Earle V. Roberts[1]

[1] Department of Chemistry; [2] Department of Basic Medical Sciences, University of the West Indies, Mona, Kingston 7, Jamaica

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Abstract: The present study reports on the insecticidal and acaricidal potentials of dibenzyltrisulfide (DBTS) isolated from the roots of *Petiveria alliacea* L. using thin layer and high performance liquid chromatography.

The 96-h LD_{50} value (μg per tick) obtained for adult *Boophilus microplus* (Canestrini) topically treated with DBTS was 0·920. The LD_{50} values obtained for three commercial acaricides dimethoate, lindane and carbaryl were 4·6, 9·3 and 6·9 μg per tick respectively. The IOD_{50} and IHD_{50} (concentrations inhibiting egg laying and hatching by 50% respectively) in μg per tick doses for DBTS were 0·22 and 0·24 respectively.

The 24-h LD_{50} dose (μg per insect) obtained for DBTS on adult *Cylas formicarius elegantulus* (Summer) was 0·193 μg per insect. The vapour from a stock solution of 5 g litre^{-1} of DBTS was highly toxic to adult *Hypothenemus hampei* Ferr. inside coffee berries, inflicting 89% mortality within 24 h.

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1 INTRODUCTION

Efforts to alleviate the economic losses inflicted by insect pests on the world's food production have led to the widespread use of synthetic organochlorine and organophosphorus insecticides. These synthetic insecticides have created many ecological problems, chief among them being toxicity to non-target organisms and development of resistance by various insect species.[1,2] These problems have compelled the applied entomologists and agricultural chemists to search for environmentally safer pesticides.

In order to address the above-mentioned problems, the efficacy of various plant extracts and compounds has been evaluated against several pest species. The compounds isolated from two of these plants, namely *Chrysanthemum cinerariefolium* (Trev) and *Physostigma venenosum* have served as the prototype molecules for the synthesis of the pyrethroid and carbamate insecticides.[3] Thus, it is important for researchers to continue the search for these biologically active plant molecules which could form the basis for the synthesis of more effective and ecologically safer pest management agents. It is along these lines that the present research was instigated.

Williams and Mansingh[4] have revealed moderate insecticidal activity in a crude ethanol extract from the roots of guinea hen weed, *Petiveria alliacea* L. (Phytolaccaceae) against *Tribolium confusum* (Duval). In the present paper the authors have isolated and characterized one of the major insecticidal and acaricidal compounds from the roots of *P. alliacea*.

2 CHEMISTRY

Infra-red spectra were determined using a Nicolet 7199 FT-IR spectrometer. Mass spectra were obtained at an ionizing voltage of 70 eV on a Krotos MS-50 (high-resolution) AE1 instrument. A Finnigan model 4000 mass spectrometer was used for low resolution mass

* To whom correspondence should be addressed.

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measurement (70 eV, EI). Nuclear magnetic resonance spectra were recorded on a Bruker AM-300 instrument operating at 300·133 MHz (^{1}H) and 75·469 MHz (^{13}C). Deuterochloroform was used as solvent and the internal standard was tetramethylsilane. A Jeol 60 MHz spectrometer was used for low field NMR measurement.

2.1 Isolation procedures

The milled roots of *Petivera alliacea* (1·4 kg) were extracted using ethanol (2·8 litre), to afford 65 g of a yellowish-brown gum. The gum was then dissolved in acetone (400 ml) and the residue redissolved in ethanol (20 ml). The ethanol-soluble portion was chromatographed over silica gel using a solvent system of cyclohexane + ethyl acetate (8 + 2 by volume). Several 20-ml fractions were collected and pooled after being screened by TLC. Thus flasks 4–9, 10–12, 13–20 and 21–23 were pooled to give four main fractions: A, B, C and D, respectively.

Fraction A, the major insecticidally active fraction was chromatographed over silica gel using a 7924T chromatotron. The chromatographic plate (2 mm) was activated in an oven at 80°C for 40 min prior to use. Nitrogen was used to purge the chromatotron after which the plate was equilibrated with the mobile phase (cyclohexane + ethyl acetate, 9 + 1 by volume), at a flow rate of 6·0 ml min^{-1} for 10 min. Elution of the fractions was monitored using a UV lamp at 254 nm. Three fractions were collected of which fraction No. 1. was the most biologically active. This fraction was purified by normal phase HPLC, using hexane + chloroform (97 + 3 by volume) as the mobile phase at a flow rate of 25 ml min^{-1}. The fraction eluting at 5·0 min showed significant insecticidal activity and afforded dibenzyltrisulfide.

Dibenzyltrisulfide was isolated as a colourless, viscous oil (74 mg, 0·005% yield); found M^+ 278·0257, $C_{14}H_{14}S_3$ requires 278·0257. (See Fig. 1).

2.2 Elucidation

The [^{1}H] NMR spectrum showed resonances at δ4·0 and δ7·25 with an integration ratio of 2 to 5. The signal at δ7·25 was attributed to protons on aromatic rings. The other signal occurring at δ4·0 was attributed to methylenic protons.

The mass spectrum revealed a base peak fragment at m/z 91. This is diagnostic for the tropylium ion which is a classical feature of benzene rings having vicinal methyl, methylene or methine substituents. The molecular peak at m/z 278 is consistent with that reported for the molecular formula of $C_{14}H_{14}S_3$. Thus, it would appear that the benzene ring was substituted by methylenic groups, which suggests that the sulfur atoms could be situated between the rings.

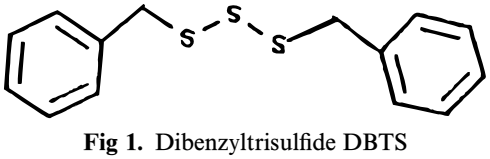

Fig 1. Dibenzyltrisulfide DBTS

The important ionization peaks (m/z) found for this compound were 280 $[M + 2]^+$, 0·4; 278 $[M]^+$ $C_{14}H_{14}S_3$, 246 $[M\text{-}S]^+$, 5 $C_{14}H_{14}S_2$; 213 (7) $C_{13}H_{13}S$, 123 (9) C_7H_7S, 91 (100-base peak) C_7H_7, 77 (13), 65 (40) C_5H_5, 51 (16). The IR data (ν max, cm^{-1}) found were 3035, 3030, 2850, 1608, 1490, 1450, 760. These data were consistent with those published by Desousa[5] for the same compound isolated from *P. alliacea.*

3 BIOLOGICAL TESTING

3.1 Test organisms

3.1.1 Ticks

Fully engorged female *Boophilus microplus* (Canestrinii) weighing between 100 and 120 mg were collected from cattle and used for bioassay within 4 h.

3.1.2 Insects

Two-week-old unsexed adult *Cylas formicarius elegantulus* (Summer) weighing 46 (±2·9) mg were used for contact insecticidal studies. Insects were cultured in glass aquaria on sweet potato tubers at 37°C and 70–80% RH.

3.1.3 Fumigation

Ripe coffee (*Coffee arabica* L. var *typica*) berries infested with adult *Hypothenemus hampei* (Ferrari) 'coffee borer' were collected from a farm located in the Blue Mountain coffee belt in the parish of St. Andrew (Jamaica).

3.2 Bioassays

3.2.1 Contact acaricidal studies

Pure samples of dibenzyltrisulfide and the three commercial acaricides (dimethoate, gamma-HCH and carbaryl) were dissolved in acetone to produce stock solutions of 0·1 to 5 g litre^{-1}. The concentrations of commercial acaricides were prepared on the basis of active ingredients (AI). Concentrations ranging between 0·005 and 10·0 μg (0·5 to 4 μl) were topically applied to the dorsa of adult *B. microplus.* The control ticks were treated with 6·0 μl of acetone in 2-μl aliquots. Thirty ticks were used in all tests in three replicates of 10 each.

The treated and control ticks were kept at 27 ± 3°C and 60–70% RH. Mortality data were recorded after 96 h and LD_{50} values (concentration of compound killing 50% of test organisms) calculated by probit analysis.[6] The IOD_{50} value (dose which inhibited oviposition by 50%) was also calculated, using the reduction in weight of eggs produced during the 14-day ovarian cycle.

TABLE 1
Ninety-Six-Hour LD_{50} and LD_{95} Values of Dibenzyltrisulfide and Commercial Acaricides for Fully Engorged Female *Boophilus microplus*

Compound	LD_{50}	(*95% FL*)	LD_{95} (*95% FL*) (*μg per tick*)
Dibenzyltrisulfide	0·918	(0·510–1·374)	18·628 (10·019–24·77)
Dimethoate	4·50	(2·132–6·5)	13·56 (12·63–15·07)
γ-HCH	9·33	(7·243–11·7)	23·44 (18·67–30·01)
Carbaryl	6·90	(4·544–9·3)	18·94 (15·43–24·62)

The inhibition of oviposition (IO) was calculated by

$$IO = \frac{\text{Mean control egg wt.} - \text{mean treated egg wt}}{\text{mean control egg weight}} \times 100$$

The viability of eggs oviposited by the treated ticks at each concentration was determined by pooling the eggs from all replicates for a particular concentration after 14 days and incubating them in test tubes plugged with moist cotton and kept at 27(±2)°C and 70–80% RH. The cotton plug was moistened periodically with distilled water. After eight weeks of incubation, the percentage of hatching was determined by removing the hatched larvae from the top of each test tube (where they crawled up and aggregated), and analysing the residue at the bottom for unhatched eggs. Three subsamples of the residues containing 200–300 eggs or eggshells were drawn from each tube, spread on a glass slide and examined under a microscope at 100 × . The ratio of hatched egg shells to unhatched eggs was used in determining the inhibition of hatching as shown in the equation below. The data thus collected enabled the calculation of IHD_{50} value (dose causing 50% inhibition of hatching).

IH = % of unhatched eggs in control *minus* % in treated.

The trisulfide isolated from *P. alliacea* was bioassayed at its IOD_{50} dose after three weeks storage in acetone at 4°C to assess its stability.

TABLE 2
Effects of Dibenzyltrisulfide and Three Commercial Acaricides on the Oviposition of *Boophilus microplus* Eggs

	IOD_{50} (*95% FL*)	IOD_{95} (*95% FL*) (*μg per tick*)
Dibenzyltrisulfide	0·221 (0·136–0·283)	2·065 (0·431–4·02)
Dimethoate	0·290 (0·17–0·354)	0·523 (0·35–0·96)
γ-HCH	1·672 (1·06–2·523)	4·222 (2·87–6·77)
Carbaryl	6·452 (5·33–8·873)	12·45 (9·66–15·3)

3.2.2 Acaricidal stability

Ticks were topically treated with a dose of dibenzyltrisulfide equivalent to its IOD_{50} value and adult mortality, inhibition of oviposition and inhibition of hatching determined as described above.

The data obtained for adult mortality, inhibition of oviposition and hatching were determined initially (day 0) and 27 days after storage at 4°C. The differences between the two sets of data were computed and the cumulative sum of their differences divided by 27 to obtain the 'loss in bioactivity per day' (LBPD)

$$LBPD = \frac{(Cm_0 - Cm_{27}) + (IO_0 - IO_{27}) + (IH_0 + IH_{27})}{27}$$

Cm = Corrected mortality

IH = Inhibition of hatching

IO = Inhibition of oviposition

3.2.3 Contact insecticidal studies

A pure sample of dibenzyltrisulfide isolated from *P. alliacea* was dissolved in acetone to produce 5 g litre^{-1} stock solution. Concentrations ranging from 0·05 to 1·0 μg (1·0 to 2·0 μl) were topically applied to the ventral surface of the abdomen of adult *C formicarius* using a Hamilton microapplicator. The control insects were treated with 4 μl of acetone only on the ventral surface of the abdomen. Thirty insects were used in three replicates of 10 each. The controls also had 30 insects. Mortality was recorded 24 h later and data subjected to probit analysis to determine LD_{50} and LD_{95} values.

TABLE 3
Effects of Dibenzyltrisulfide and Three Commercial Acaricides on the Embryogenesis of *Boophilus microplus*

	IED_{50} (*95% FL*)	IED_{95} (*95% FL*) (*μg per tick*)
Dibenzyltrisulfide	0·236 (0·14–0·3030)	2·158 (0·451–4·219)
Dimethoate	1·100 (0·85–1·67)	2·54 (1·330–3·950)
γ-HCH[a]	—	—
Carbaryl	11·80 (9·33–19·65)	20·7 (17·52–38·97)

[a] Eggs failed to hatch in this treatment.

TABLE 4
Changes in the Activity of IOD_{50} Dose of Dibenzyltrisulfide (0·22 μg per Tick) after 27 Days in Acetone

Biological activity	*Days after storage in acetone*	
	Day 0	*Day 27*
Corrected mortality (%)	22·3	16·7
Mean egg weight (mg)	60·3 (±1·2)	40·2 (±3·3)
Inhibition of oviposition (%)	50·0	27·80
Inhibition of embryogenesis (%)	75·6	23·4
LBPD[a] for DBTS = 2·90 per day.		
Control		
Mean egg weight (mg)	80·0 (±2·40)	80·55 (±6·32)
Mean of eggs failed (%) to hatched in control	10·5 (±2·4)	9·04 (±3·50)

[a] LBPD = loss in biological activity per day (Section 3.2.2).

3.2.4 Fumigation toxicity studies
The following concentrations: 0·1, 0·25 and 5·0 g $litre^{-1}$ of dibenzyltrisulfide were prepared in acetone. Fifteen infested coffee berries were submerged in test solutions for 2 s. The control berries were dipped in acetone only. The treated berries were kept at 27(±2)°C and 55–60% RH in Petri dishes covered with muslin. After 24 h the numbers of dead borers found in Petri dishes and dissected berries were counted.

4 RESULTS AND DISCUSSION

Dibenzyltrisulfide (DBTS) was more effective in killing adult female *Boophilus microplus* than the three commercial acaricides: dimethoate, gamma-HCH and carbaryl (Table 1). Thus, the order of the 96-h LD_{50} doses (μg per tick) was: DBTS (0·918) > dimethoate (4·50) > carbaryl (6·90) > gamma-HCH (9·33). These results strongly suggest that DBTS could be a potential control agent for cattle ticks.

TABLE 5
Activity of Dibenzyltrisulfide against *Cylas formicarius*

LD_{50} (95% FL)	LD_{95} (95% FL)
(μg per insect)	
0·123 (0·101–0·152)	0·497 (0·323–0·658)

The order of inhibition of oviposition of compounds was similar to that obtained for adult mortality, except that gamma-HCH was more effective than carbaryl (Table 2). Thus, the order of activity of the compounds as judged by their IOD_{50} values was: DBTS (0·221) > dimethoate (0·290) > gamma-HCH (1·672) > carbaryl (6·452).

All compounds were effective in reducing the hatching success of eggs oviposited by the treated ticks. The order of inhibition of hatching was similar to that obtained for oviposition. Eggs oviposited by ticks treated with gamma-HCH failed to hatch, thus, highlighting its potent ovicidal action as compared to the other test materials (Table 3).

Dibenzyltrisulfide lost 2·9% of its acaricidal potency per day (Table 4), thus over a 27-day period the compound lost 78·30% of its activity. This result is interesting and deserves investigation under field conditions, since farmers would like to use compounds which are readily biodegradable in their pest management programmes in order to prevent contamination of the environment. Similarly, the toxicity of DBTS should be fully explored on non-target aquatic and terrestrial organisms in order to evaluate its broad-spectrum action.

Tables 5 and 6 reveal that the trisulfide isolated from *P. alliacea* has potent contact insecticidal and fumi-

TABLE 6
Control of *Hypothenemus hampei* after Dipping of Infested Coffee Berries into Various Concentrations of Dibenzyltrisulfide

Concentration of DBTS (g $litre^{-1}$)	0 (acetone control)	0·1	0·25	5·0
24-h mortality (%)	0·0	46·2	59·6	89·0

gation actions against *C. formicarius* and *H. hampei* respectively. The LD_{50} value of the trisulfide against *C. formicarius* was 0·123 (0·101–0·152) μg per insect (slope = 7·53). The compound was also very effective in killing *H. hampei* inside coffee berries at all concentrations tested (Table 6). Further research is required at the field level in order to assess the efficacy of dibenzyltrisulfide against *H. hampei* which is a major problem in the coffee-growing regions of the world.

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